CENTRE FOR
CRYSTALLOGRAPHIC
STUDIES

Department of Chemistry, University of Copenhagen

Projects

1. Studies of the structure and function of proteins
    1. Enzymes involved in the nucleotide metabolism
    2. Enzymes involved in the degradation and synthesis of complex carbohydrates
        1. Pectin degrading enzymes
        2. Enzymes in clan GH-A
        3. Starch modifying enzymes
    3. Heme containing proteins
    4. Condensing enzymes in the biosynthesis of fatty acids
    5. Upcoming projects

2. Interactions between chiral molecules, structural and thermodynamic studies

3. Properties derived from the electron density of molecules and crystals

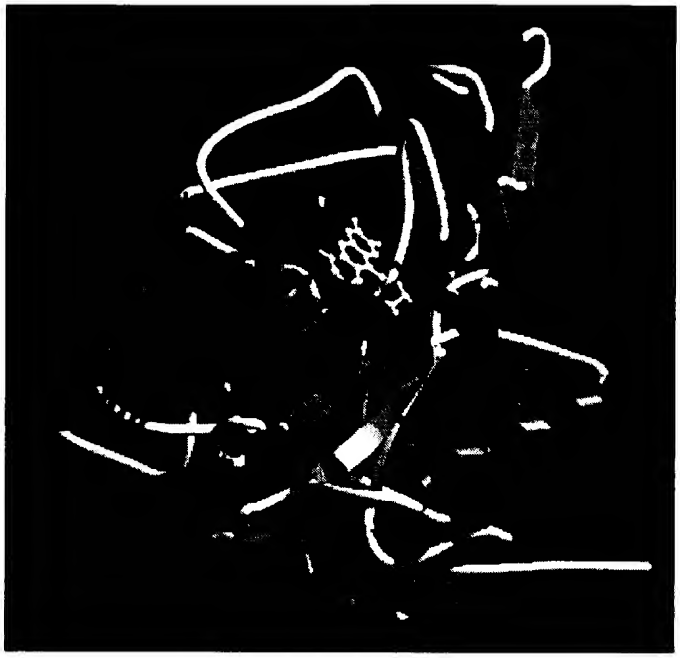
X-ray diffraction studies constitute the primary experimental method in these projects, but it is frequently supplemented with other physico-chemical measurements like differential scanning calorimetry and spectroscopy.

1. Studies of the structure and function of proteins

1.1. Enzymes involved in the nucleotide metabolism

We are investigating the structure and function of several of the enzymes that are involved in the metabolism of nucleotides in collaboration with the group of Prof. Kaj Frank Jensen, University of Copenhagen. The proteins studied are recombinant enzymes mainly of bacterial origin. We investigate primarily some of the enzymes that are involved in the *de novo* synthesis of UMP, uridine monophosphate: dihydroorotate dehydrogenases, orotidine monophosphate decarboxylase, phosphoribosyl pyrophosphate synthetases, uracil phosphoribosyl transferases and uridine-cytodine kinases.

Another project focuses on RNase PH, an exoribonuclease involved in the processing of tRNA precursors from the 3'-terminus of tRNA.



Dihydroorotate dehydrogenase A has an alpha beta-barrel structure. The active site of this enzyme is above the flavin co-factor (yellow).

Our investigations of the different enzymes are in quite different stages, some have their three dimensional structure well characterised and a profound understanding of their enzymatic function is within close reach (dihydroorotate dehydrogenases, phosphoribosyl pyrophosphate synthetases and uracil phosphoribosyltransferases). The structure of RNase PH has recently been determined and awaits a thorough analysis that would lead to important insight into the unique role this enzyme plays in the degradation of tRNA. The other projects are in a more preliminary stage, some enzymes have been crystallised and we are on the way with their structure determination.

The hard work associated with the projects described above are being conducted by the following members of the group, Sofie Norager (dihydroorotate dehydrogenases), Tine Astrup Eriksen and Frank Nygaard (phosphoribosyl pyrophosphate synthetaseses), Anders Kadziola (uracil phosphoribosyl transferases and RNase PH), Anette Frost Jensen (uridine cytidine kinase). Finally Jens-Christian Navarro Poulsen and Pernille Harris are responsible for the orotidine decarboxylase project.

Publications:

[5] Overexpression of *Bacillus subtilis* Phosphoribosylpyrophosphate Synthetase and Crystallization and Preliminary X-Ray Characterization of the Free Enzyme and Its Substrate-Effector Complexes.

[6] Purification and characterization of dihydroorotate dehydrogenase A from *Lactococcus lactis*, crystallization and preliminary X-ray diffraction studies of the enzyme.

[8] The crystal structure of the flavin containing enzyme dihydroorotate dehydrogenase A from *Lactococcus lactis*.

[11] The active site of dihydroorotate dehydrogenase A from *Lactococcus lactis* investigated by chemical modification and mutagenesis.

[15] The crystal structure of *Lactococcus lactis* dihydroorotate dehydrogenase A complexed with the enzyme reaction product throws light on its enzymatic function.

1.2. Carbohydrate modifying enzymes

1.2.1. Pectin degrading enzymes

One group of projects has focused on enzymes involved in pectic substance degradation. Most of the work has so far been carried out on enzymes acting on **rhamnogalacturonan**, the hairy region of pectin. **Rhamnogalacturonan** I (RG-I) has a backbone composed of alternating rhamnose and galacturonic acid residues which can be acetylated and methylated. The rhamnose residues of the backbone usually have galactan, arabinan or arabinogalactan attached to C4 as side chains. We have determined the structures of several of the enzymes that take part in the enzymatic degradation of RG-I. Rhamnogalacturonase A (Rgase A) (1RMG) an endoacting hydrolase cleaving the 1,2 glycosidic bonds within the backbone of RG-I, **rhamnogalacturonan** acetylesterase (RGAE) (1K7C, 1DEO, 1DEX) which works in synergy with RGases removing the acetylesters from the backbone, and a beta-1,4-galactanase (1FHL, 1FOB, on hold) hydrolysing the glycosidic bonds in galactan side chains. Very recently, the structure of **rhamnogalacturonan** lyase (previously known as RGase B), which cleaves the (alpha-1,4 glycosidic linkage of RG-I, has been solved and is being analysed. Among projects in a less advanced stage that can be mentioned is a beta-1,4-mannanase.

Many of these enzymes are glycosylated, and the influence of the glycosylation on the crystallisation and function of the enzymes represents a very interesting aspect (see *eg.* the Rgase A [9] and RGAE [13] [46] papers).

The smooth region of pectin is composed of unbranched polygalacturonan. The galacturonic acid residues can be methylated at the carboxylate group or acetylated at the C2 or C3 position. The backbone of polygalacturonan is broken down by pectin and pectate lyases. Pectate lyase (Pel) from *Bacillus agaradherens*, *Bacillus licheniformis*, and *Thermatoga maritima* have recently been solved and are currently being analysed. Complexes of two separate inactive mutants of *Thermatoga maritima* Pel with a galacturonic acid oligomer of five residues have recently been obtained. These complexes provide added insight into the substrate recognition and catalytic mechanism of Pels. Structural features that confer themostabilty to the *Thermatoga maritima* Pel are also being investigated. Pectate backbone degradation by Pels is favored after the removal of acetyl groups, which is catalyzed by pectin acetylesterase (PaeY). We are currently optimizing the crystallization conditions for PaeY.

We aim to understand the catalytic mechanism for these enzymes. Most of these enzymes are isolated from *Aspergillus aculeatus* and cloned and expressed in *Aspergillus oryzae* by Novozymes A/S. The people who have been working on these projects are [illegible] (RGAE and PaeY), [illegible]

Publications

[9] The crystal structure of rhamnogalacturonase A from *Aspergillus aculeatus*: a right-handed parallel beta helix.

[13] Crystallization and preliminary X-ray diffraction studies of the heterogeneously glycosylated enzyme **rhamnogalacturonan** acetylesterase from *Aspergillus aculeatus*.

[30] **Rhamnogalacturonan** acetylesterase elucidates the structure and function of a new family of hydrolases.

[46] A branched N-linked glycan at atomic resolution in the 1.12 A structure of **rhamnogalacturonan** acetylesterase.

[50] Crystallization and preliminary X-ray characterization of a thermostable pectate lyase from *Thermotoga maritima*.

[55] A stepwise optimization of crystals of **rhamnogalacturonan** lyase from *Aspergillus aculeatus*.

1.2.2. Enzymes in clan GH-A

Another group of projects focusses on the structural determinants of polysaccharide specificity in glycoside hydrolases belonging to the 4 7 superfamily clan GH-A, a group of enzymes with conserved 8-fold beta alpha-barrel architecture, conserved mechanism but different substrate specificities. The structure of the first fungal family 5 endoglucanase has recently been determined and shed new light in the role of a conserved Gly (1GZJ). A thermostable family 10 xylanase has been particularly the subject of detailed investigation (1K6A, 1GOK, 1GOM, 1GOQ, 1GOR, 1GOO). In collaboration with Novozymes A S several family 53 galactanases involved in degradation of pectin side chains (see 1.2.1.) are being investigated. Leila Lo Leggio, Jerome Le Nours and Carsten Rytterstgaard have been mostly involved in these projects. One idea we are particularly interested in is the involvement of shape complementarity of polysaccharide and enzyme as a determinant of specificity, which we are exploring in our work with the enzymes in families 10 and 53 in particular.

As part of our studies of plant cell wall degrading enzymes, an unrelated family 9 glycoside hydrolase from *Alicyclobacillus acidocaldarius*, is also being investigated in collaboration with Kelvin Eckert and Prof Erwin Schneider at the Humboldt University, Berlin.

Publications

[12] Crystallization and preliminary X-ray analysis of the major endoglucanase from *Thermoascus aurantiacus*.

[redacted] Xylanase I: implications for the

evolution of thermostability in family 10 xylanases and enzymes with beta alpha-barrel architecture.

[26] Xylanase-oligosaccharide interactions studied by a competitive enzyme assay.

[33] X-Ray Crystallographic Study of Xylopentaose Binding to *Pseudomonas fluorescens* Xylanase A.

[37] Anisotropic refinement of the structure of *Thermoascus aurantiacus* xylanase I.

[44] Substrate specificity and subsite mobility in *Thermoascus aurantiacus* xylanase 10A.

1.2.3. Starch modifying enzymes

One member of the group, Anders Kadziola, has previously been involved in structural studies of barley amylase. In the last few years the group has also been involved in collaborations with Danisco Cultor Innovations on several enzymes which are involved in starch or glycogen modification in vivo or biotechnological processes. Projects include modelling and crystallographic studies of branching enzymes and maltose O-acetyltransferase. Recently the group has started a wider collaboration in this area within the EU funded NEPSA project. Leila Lo Leggio and Heidi A. Ernst are mostly working in this area.

Publications

[2] Crystal and Molecular Structure of Barley alpha-Amylase.

[14] Barley alpha-amylase bound to its endogenous protein inhibitor BASI: crystal structure of the complex at 1.9 A resolution.

[17] Molecular Structure of a Barley alpha-Amylase-Inhibitor Complex: Implications for Starch Binding and Catalysis.

[32] Characterisation and Crystallisation of an active N-terminally Truncated Form of the *Escherichia coli* Glycogen Branching Enzyme.

[42] Crystallization and preliminary X-ray analysis of maltose O-acetyltransferase.

[53] A structural model for the N-terminal N1 module of *E. coli* glycogen branching enzyme.

1.3. Heme containing enzymes

Structural studies of heme containing enzymes were among our first protein crystallographic projects and have led to the determination of the three dimensional structure of the peroxidase from the fungus *Coprinus cinereus* (1LYS, 1LY9, 1LYC, 1LYK) and the cytochrome c4 from *Pseudomonas stutzeri* (1EFP). The study of the former enzyme is part of a collaborative project with Novozymes A/S, and the latter protein with Prof. Jens Ulstrup's group from the Technical University. Presently we focus on structure determinations for the oxidised forms of the peroxidase and on a collaborative project with Prof. Birger Lindberg Moller's group from the Royal Veterinary and Agricultural University on structure determinations for cytochromes P450 involved in plant biosynthesis. The work on the *Coprinus* peroxidase is primarily conducted by Pernille Harris and Karen Houborg.

The electrostatic potential mapped onto the molecular surface with direction of the dipole moment shown (green arrows). Blue represents positive charge distribution and red negative charge. The view is along the pseudo-twofold axis: front view (left) and back view (right). [6]

Publications

[1] Three-dimensional structure of a recombinant peroxidase from *Coprinus cinereus* at 2.6 Å resolution.

[3] Crystallization and preliminary crystallographic investigations of cytochrome c_4 from *Pseudomonas stutzeri*.

[7] Crystal structure of the dihaem cytochrome c_4 from *Pseudomonas stutzeri* determined at 2.2Å resolution.

1.4. Condensing enzymes in the biosynthesis of fatty acids

In collaboration with Penny von Wettstein-Knowles' group at the Institute of Molecular Biology, University of Copenhagen we are studying one of the enzymes that catalyse the condensing step in the biosynthesis of fatty acids, beta-keto-acyl (acyl carrier protein) synthetases (KAS I) from *E. coli*.

Johan Gotthardt Olsen is performing these investigations. The crystal structure of KAS I has been solved to 2.3 Å and the fold is similar to that of KAS II [illegible]

Publications

[4] Preliminary X-ray Diffraction Analysis of beta-Ketoacyl-[Acyl Carrier Protein]Synthase I from *Escherichia coli*.

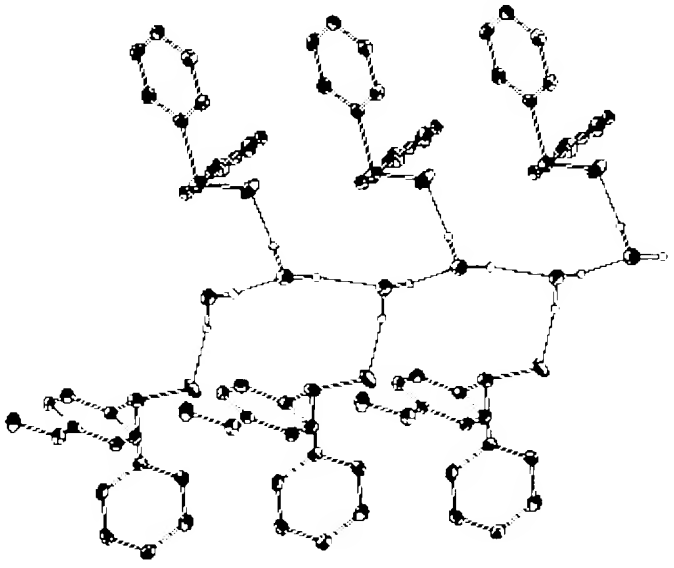
[21] The X-ray crystal structure of beta-ketoacyl [acyl carrier protein] synthase I

1.5. Upcoming projects

Structural investigations of plasma membrane proton pumps will bring new challenges to the group. Their primary role is in the coupling of metabolic energy to solute transport. It is collaborative project that involve several European research groups and is supported partly by a grant from the European Union. The groups led by Prof. Marc Boutry, Catolique University of Louvain, Belgium and Prof. Michael Palmgren, The Royal Veterinary and Agricultural University represent our primary collaborators.

2. Structural and thermodynamic studies of interactions between chiral molecules

The interactions between chiral molecules are important for most biological processes. The enantiomers of a chiral molecule would frequently lead to different biological functions and so it is important to use the pure enantiomers as drugs, herbicides and pesticides. Most of the synthetic routes that are used in their preparation do not lead to pure enantiomers but to a racemic mixture. Therefore it has become increasingly important to understand the physico-chemical and structural background for the processes that are used to isolate the pure enantiomers. We have approached this by studying two principally different types of problems. One where we investigate the thermodynamic and structural aspects of the crystallisation of racemates, and in the other where we study the resolution of racemates through [illegible]



part of Katalin Marthi's Ph.D. project. The Ph.D. student Henning O. Sorensen is also involved in these studies.

Publications

[1] Crystal Structure of (*S*)-1-Phenylethylammonium (*R*)-Mandelate and a Comparison of Diastereomeric Mandelate Salts of 1-Phenylethylamine.

[2] Structures with Identical Packing; Racemic and Partially Optically Pure 3-(2'-Chloro-2'-phenylethyl)-2-thiazolidiniminium *p*-Toluenesulfonate and a Comparison of the Packing in Corresponding Racemic and Optically Active Compounds.

[3] The Role of Solvates in Optical Resolution. A Study of the Diastereoisomeric Salts Formed From Enantiomeric 2-Amino-2-phenylethanol and (*R*)-Mandelic Acid, their Crystal Structures and Physico-chemical Properties.

[4] Structures of Racemic Monofluoro-Substituted Mandelic Acids, Their Relation to the Thermochemical Properties and an Analysis of Short Intermolecular Fluorine-Carbon Contacts.

[5] Structures of Racemic Halogen-Substituted 3-Hydroxy-3-phenylpropionic Acids; Relations Between Spontaneously Resolved and Racemic Compounds.

[6] Structures of the Optically Active Monofluoro-Substituted Mandelee Acids: Relation to Their Racemic Counterparts and Thermochemical Properties.

[7] A Study of Diastereomeric Mandelic Salts of Cinchonidine and the Relation to Their Quasidiastereomeric Analogues.

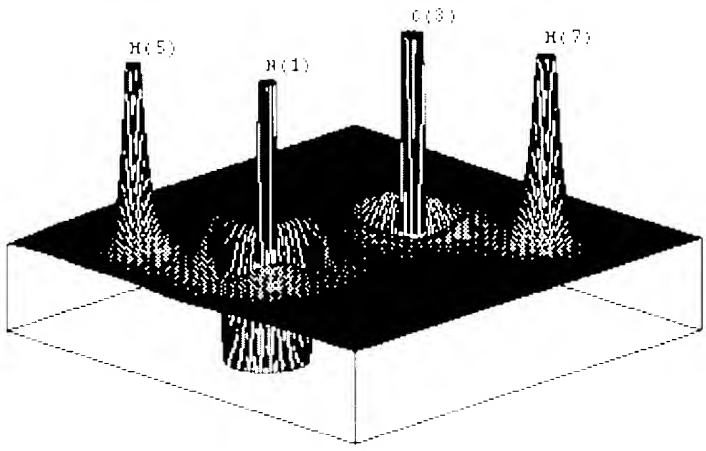
[8] Structural and Thermodynamic Relationships Between Optically Active and Racemic Compounds. The Crystal Structures of Optically Active Chloro- and Bromo-Substituted 3-Hydroxy-3-phenylpropionic Acids.

[9] Optical Resolution of 2-(2'-Hydroxyethylamino)-1-phenylethanol, and the Crystal Structures of Two Polymorphic Modifications of the (2*R*,3*R*)-*O'*-Dibenzoyl Hydrogen Tartrate Salt of the (*S*)-(+)-Enantioner.

[10] Quininium (*S*)-mandelate.

3. Properties derived from the electron density of molecules and crystals

We use the information that the X-ray diffraction data contain about the thermally averaged electron density to study interatomic interactions in crystals. These investigations require very accurate high resolution X-ray diffraction data. Frequently a complementary neutron diffraction study is performed to get accurate parameters for the hydrogen atoms in the crystals. The collaboration with professor Robert F. Stewart, Carnegie-Mellon University, plays an important role for these investigations, which also involve a significant amount of program development for the program system VALRAY, which is primarily used for the computations involving the diffraction data. The topological analysis developed by R. Bader for theoretical electron densities is successfully employed to study the properties of the experimental electron densities.



Negative Laplacian of the crystal electron density ($-\nabla^2(\rho)$) in the mirror plane of the methylammonium cation. The positive values are truncated at 256 e A^5 and negative 128 e A^5. Atoms in the plane are H(5), N(1), C(3) and H(7). [1]

It has been natural to complement these investigations with comparative quantum chemical calculations both on the molecular entities and the crystal. For the crystals we primarily use periodic Hartree-Fock calculations (CRYSTAL95).

The systems studied are molecular crystals that display interesting interatomic interactions.

An example is the recently completed research on the very short hydrogen bonds. We are exploiting the potential of these methods to include acentric crystals and are extending this work to the study of weaker intermolecular interactions. These investigations were the topic of Claus Flensburg's Ph.D. thesis, and Dennis Madsen was also involved in some of the studies. Ph.D. student Henning O. Sorensen, and cand. scient. Annette Langkilde have likewise been working with these projects.

Publications

[1] Experimental Charge Density Study of Methylammonium Hydrogen Succinate Monohydrate. A Salt with a Very Short O-H-O Hydrogen Bond.

[2] Properties of the Experimental Crystal Charge Density of Methylammonium Hydrogen Maleate. A Salt with a Very Short Intramolecular O-H-O Hydrogen Bond.

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